

## I Claim

1. A cell free extract from Ciliate phylum microorganism, wherein said cell free extract contains cholesterol desaturase activities selected from the group comprising  $\Delta$ -7 and  $\Delta$ -22 cholesterol desaturases activities that catalyze desaturation of cholesterol.

2. A cell free extract of Claim 1, wherein said cell free extract is selected from the group consisting of cell free homogenate, microsomal fraction and desaturase-enriched fraction, or a combination thereof, all from Ciliata phylum microorganism.

3. A cell free extract of Claim 1, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

4. A process for manufacturing  $\Delta$ 7 dehydrocholesterol (provitamin D3) and  $\Delta$  7,22 bis dehydrocholesterol comprising:

(a) mixing a cell free extract of claim 1 with a cholesterol substrate;

(b) incubating the mixture for a period of time enough to produce  $\Delta$ 7 dehydrocholesterol and  $\Delta$ 7,22 bis dehydrocholesterol;

(c) recovering said  $\Delta$ 7 dehydrocholesterol and  $\Delta$ 7,22 bis dehydrocholesterol by solvent extraction and chromatographic purification.

5. A substantial pure  $\Delta 7$  cholesterol desaturase enzyme from Ciliata phylum microorganism, wherein said enzyme is capable of catalyzing the conversion of a cholesterol substrate in  $\Delta 7$  dehydrocholesterol by introducing a double bond at the position seven in the cholesterol molecule.

6. A substantial pure  $\Delta 7$  cholesterol desaturase enzyme of Claim 5, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

7. A substantial pure  $\Delta 7$  cholesterol desaturase enzyme according to claim 5, the enzyme

(a) having a molecular weight of approximately 60 kDa by gel chromatography;

(b) having an optimum pH range for enzymatic activity between 6.5-8.5;

(c) having an optimum temperature range for enzymatic activity of 28°C to 35°C;

(d) being unaffected by metal ions such as  $\text{Ca}^{+2}$ ,  $\text{Mn}^{+2}$  and  $\text{Mg}^{+2}$ , EDTA concentrations and 2-mercaptoethanol;

(e) being inactivated after 1 minute at 100°C;

(f) being storage at -20°C by at least 6 months.

8. A substantial pure  $\Delta 22$  cholesterol desaturase enzyme from Ciliata phylum microorganism, wherein said enzyme is capable of catalyzing the conversion of a cholesterol substrate in  $\Delta 22$

dehydrocholesterol by introducing a double bound at the position twenty-two in the cholesterol molecule.

9. A substantial pure  $\Delta^{22}$  cholesterol desaturase enzyme of Claim 8, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

10. A substantial pure  $\Delta^{22}$  cholesterol desaturase enzyme according to claim 8, the enzyme

(a) having a molecular weight of approximately 60 kDa by gel chromatography;

(b) having an optimum pH range for enzymatic activity between 5.5-8.5;

(c) having an optimum temperature range for enzymatic activity of 28°C to 35°C;

(d) being unaffected by metal ions such as  $\text{Ca}^{+2}$ ,  $\text{Mn}^{+2}$  and  $\text{Mg}^{+2}$  and EDTA concentrations;

(e) being inactivated after 1 minute at 100°C;

(f) being storage at -20°C by at least 6 months.

11. A process for preparing a substantial pure  $\Delta^7$  cholesterol desaturase enzyme from Ciliata phylum microorganism according to claim 5, the process comprising the steps of:

(a) culturing a microorganism in a suitable medium, wherein said microorganism is capable of producing  $\Delta^7$  cholesterol desaturases;

(b) disintegrating the culture and extracting the same with buffer solution containing, if necessary, non ionic surfactant or stabilizer as glycerol;

(c) subjecting the extract to a chromatography purification under suitable conditions; and

(d) eluting and recovering said  $\Delta 7$  cholesterol desaturases.

12. The process according the claim 11, wherein the step of culturing is carried out in a medium containing 1% proteose peptone, 0.1% yeast extract, 0.5% glucose, 0.01% Sequestrene and 0,5mg% of 22 dehydrocholesterol.

13. The process according the claim 11, wherein the chromatography purification is selected from a group comprising size exclusion chromatography, anion exchange chromatography, cation exchange chromatography and combinations thereof.

14. A process for preparing a substantial pure  $\Delta 22$  cholesterol desaturase enzyme from Ciliata phylum microorganism according to claim 8, the process comprising the steps of:

(a) culturing a microorganism in a suitable medium, wherein said microorganism is capable of producing  $\Delta 22$  cholesterol desaturases;

(b) disintegrating the culture and extracting the same with buffer solution containing, if necessary, non ionic surfactant or stabilizer as glycerol;

(c) subjecting the extract to a chromatography purification on a suitable chromatography conditions; and

(d) eluting and recovering said  $\Delta^{22}$  cholesterol desaturases.

15. The process according the claim 14, wherein the step of culturing is carried out in a medium containing 1% proteose peptone, 0.1% yeast extract, 0.5% glucose, 0.01% Sequestrene and 1.0 mg% of cholesterol.

16. The process according the claim 14, wherein the chromatography purification is selected from a group comprising size exclusion chromatography, anion exchange chromatography, cation exchange chromatography and combinations thereof.

17. The use of substantial pure  $\Delta^7$  cholesterol desaturase enzyme from Ciliata phylum microorganism of claim 5 for producing  $\Delta^7$  dehydrocholesterol (provitamin D3) employing cholesterol as substrate.

18. The use according the claim 17, wherein the cholesterol substrate es seleccionado del grupo comprendido por colesterol puro, colesterol-containing products and cholesterol enriched fractions.

19. The use according the claim 17, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

20. The use of pure  $\Delta^7$  cholesterol desaturase and substantial pure  $\Delta^{22}$  cholesterol desaturase enzymes from Ciliata phylum microorganism of claims 5 and 8 for producing  $\Delta^{7,22}$  bis dehydrocholesterol employing cholesterol as substrate.

21. The use according the claim 20, wherein the cholesterol substrate es seleccionado del grupo comprendido por colesterol puro, colesterol-containing products and cholesterol enriched fractions.

22. The use according the claim 20, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.